



Evaluation of Anti-Epileptic Properties of Ashwagandha and Chamomile by PTZ-Induced Mouse Model

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Abstract: Ayurvedic treatments are used in various types of neurological disorders. One of the common neurological disorders is Epilepsy. *Withania somnifera* and *Matricaria recutita* are well known for their medicinal properties, such as antimicrobial, antioxidant, anti-inflammatory, and antiepileptic properties. There are also studies examining the properties of *Withania somnifera* and *Matricaria recutita*. This study aims to prove the antiepileptic properties of *Withania somnifera* and *Matricaria recutita* extracts by testing them on epilepsy-induced Swiss albino mice. Five groups of male Swiss albino mice of 40±5g of weight, each consisting of 5 mice. The drugs were administered at a dose level of 100 mg/kg b.wt each for a week. The inducing method was used to induce epilepsy. Pentylentetrazol (PTZ) is injected, which acts as a GABA-A receptor antagonist. It stimulates the nerves and causes seizures. The first group acted as a control, the second group as a reference control, with Diazepam, and the remaining groups were dosed with the herbal compound orally before PTZ injection (120 mg/kg b.wt). The evaluation of the latency period and the seizure period was done. The results were obtained and analyzed with ANOVA. Chamomile and Diazepam showed longer latency periods of 102±42.64 s and 103.5±20.50 s, respectively. In the seizure period, the combination group showed good results, having fewer jerks in the clonic period, less than 10 s of the tonic period, and less than 8 s recovery. This showed the anti-epilepsy activity of the herbal extracts of *Withania somnifera* and *Matricaria recutita*.

Keywords: *Withania somnifera*, *Matricaria recutita*, Epilepsy, Seizures, Mice, Pentylentetrazol.

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I. INTRODUCTION

One of the most prevalent disorders of the central nervous system is epilepsy, and uncontrolled seizures raise the risk of mortality¹. A substantial amount of the global disease burden is accounted for by epilepsy, which affects about 50 million individuals globally. Between 4 and 10 persons, out of every 1000 are reported to have active epilepsy, implying they are either still experiencing seizures or require medication (WHO 2023). In India, there are about 10 million people who have epilepsy (PWE). In our demography, it affects roughly 1% of people². Antiepileptic medications reduce seizures, but they cannot treat the underlying cause of the condition; but can only treat its symptoms. Treatment with antiepileptic medications is successful in 65% of instances. These medications prevent seizures in two ways. The first relates to medications such as benzodiazepines and lorazepam, which reduces the symptomatic seizures by enhancing GABA-AR mediated inhibition; the second relates to medications containing phenytoin and carbamazepine, which activate voltage-gated Na⁺ channels and consequently decrease potential³. However, because of their severe and unbearable adverse effects^{4,5}, their usage is restricted in many individuals. PTZ, a convulsant of the central nervous system, competitively agonists the GABA-A receptor via an allosteric interaction in the Cl⁻ channel^{6,7}. It creates seizures when administered to animals. Herbal medications are being used these days to treat any illness, including epilepsy. *Matricaria recutita* and *Withania somnifera* contain anti-epileptic and their unique therapeutic qualities^{9,10}. In this study, the anti-epileptic qualities of the herbal medications are tested in animal models, and PTZ is used to cause seizures.

1.1. *Matricaria recutita*

Matricaria recutita is commonly known as Chamomile. It grows to a height of 10 to 60 cm. It has feathery foliage with white flowers that resemble daisies. Its foliage lacks fragrance, but the flowers have fragrance¹¹. It possesses sedative, antibacterial, antispasmodic, antioxidant, anti-inflammatory, anti-anxiety^{12,13} and restorative qualities. Over 120 bioactive components are present in these herbs, with phenolic compounds being the most prominent and responsible for the antioxidant effect. Flavonoids and their derivatives are also responsible for their antibacterial and anti-inflammatory properties. Moreover, it promotes the development of tissue granulation during wound healing¹⁴. Chamomile is also used as an anti-convulsant in animal studies¹⁵.

1.2. *Withania somnifera*

Withania somnifera is an upright, branched, evergreen shrub that grows to a height of 30 to 150 cm. It is green

throughout the year and has strong-smelling roots¹⁶. It is commonly used as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, the elderly, and during pregnancy¹⁷. According to research, Ashwagandha has antioxidant, hemopoietic, anticancer, antistress¹, and immunomodulatory qualities. The central neurological, endocrine system, and heart also benefit from Ashwagandha as well¹⁸. It has neuroprotective and anti-neurodegenerative effects¹⁹. In this study, we evaluated the effect of both *Withania somnifera* and *Matricaria recutita* on their anti-epileptic property using a PTZ-induced mice model.

2. MATERIALS AND METHODS

2.1. Preparation of Herbal drugs

The herbal drugs used are *Withania Somnifera* and *Matricaria recutita*, with the common names of Ashwagandha and Chamomile. The dried powder of both drugs was purchased from Merlion Naturals, Ahmedabad, India. The powder obtained was characterized and authenticated microscopically in our facility. The powder was grounded into a coarse powder with a mortar and pestle before being blended with CMC (1.5%) to create a full suspension for oral dosage at a concentration of 100 mg/kg b.wt for both Ashwagandha and Chamomile. The Combination dose was a total of 100 mg/kg b.wt. Diazepam was utilized as a reference control²⁰.

2.2. Animals with Ethical Statement

The animal experiment was carried out as per the instructions in the protocol number MB/IAEC/2024/01/04 given by the ethical committee of Mass Biotech, Chengalpattu, with CPCSEA registration number 2084/PO/RcBt/S/19/CPCSEA. Swiss albino mice (male) of weight 40±5g were housed in polypropylene cages with stainless steel top grills, and corn cob was used as bedding material. All animals were kept in the animal room with the following environmental conditions: 23±3°C temperature, relative humidity at 50±20%, photoperiod of 12hr light/12hr dark, and the sound level was kept below 65 dB. Individual Polypropylene cages were used to house the animals with corn cob bedding. They had ad-libidum to water (RO water) and a commercially available Rodent pellet diet with 18 percent protein. The conditions were ensured as per CPCSEA regulations.

2.3. Experimental protocol

Swiss Albino mice of weight 40±5g were divided into 5 groups randomly based on their body weight. Table I lists the groups and their respective treatment and dosage of drugs.

Group	Treatment	Dose (mg/kg b.wt)	Number of animals
Group I	PTZ Control	120	5
Group II	Diazepam+PTZ	1 ²¹	5
Group III	Ashwagandha+PTZ	100	5
Group IV	Chamomile+PTZ	100	5
Group V	Combination+PTZ	100	5

Table I illustrates the different groups used in the study. Also, it has information on the different treatments, listing

the respective dosages. PTZ was administered at 120 mg/kg b.wt and Diazepam (reference control) at 1 mg/kg b.wt.

Ashwagandha, Chamomile, and Combination were administered at 100 mg/kg b.wt each. An acute toxicity study was conducted and concentration of herbal drugs was chosen for the study. Moreover, our dosage matches with the reported dosage²². Each group consists of 5 animals. The animals were dosed orally for a week. PTZ control animals were given 1.5% CMC orally, diazepam was given intraperitoneally (1 mg/kg b.wt), and the rest of the groups were dosed with their respective formulations. On the 8th day, all animals were tested for epilepsy. The animals were dosed 30 minutes before the induction of epilepsy. PTZ (120mg/kg b.wt^{23,24}) is used for the induction of seizures. PTZ was purchased from SRL Chemicals. It is dissolved in 0.9% saline, and based on the body weight of animals, PTZ was administered intraperitoneally (IP) to each animal. Every animal was examined individually after administering PTZ for 7 min.

2.4. Seizure recording

The seizure recording was based on these parameters: 1) Latency period (Time between administration of PTZ and first jerk²⁰), 2) Clonic period (period of repeated clonic jerks of the forelimbs and hindlimbs with loss of the righting reflex²⁵), 3) Tonic period (falling on the side followed by forelimb and hindlimb tonic contractions²⁶), 4) Postictal depression (the period between the end of tonic seizure to the normal stage of mice). The end of postictal depression indicates the end of a full cycle of seizures. After the administration of PTZ, the convulsive activity of mice was

recorded for 7 minutes and used in further interpretations. The evaluation is based on only two parameters, i.e., Latency and seizure period (combination of the clonic period, tonic period, and postictal depression).

2.5. Statistical analysis

The results obtained from the seizure recording were subjected to statistical analysis by using Graph pad Prism 10. The data were compared with 2-way ANOVA for significance (95% confidence interval)

3. RESULTS

On the 8th day of dosing, after 30 minutes, PTZ was administered to the animals, and each animal was subjected to a seizure recording individually for 7 minutes.

3.1. Latency period

Regarding the latency duration, we can observe from Figure 1 that the treatment groups had longer latency times, which suggests that the seizures are delayed. Comparing the Chamomile group (102.2 s) to the reference compound, diazepam (103.5 s), yields the same result. Thus, among the treatment groups, the chamomile groups have superior outcomes during the latency period. Regarding the latency duration, we can observe from Figure 1 that the treatment groups had longer latency times.

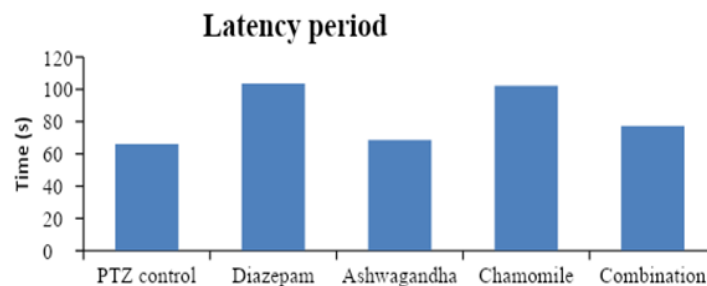


Fig 1: Latency period (the time between the administration of PTZ and the first jerk)

Figure 1 is the graphical representation of the latency period (the time between the administration of PTZ and the first jerk). It showed that Diazepam and Chamomile have longer latency periods, and all treatment groups show good results compared with the PTZ control group.

3.2. Seizure period

When comparing the treatment groups to the PTZ control, it is clear that they performed quite well during the seizure period. The combination group performs the best when all three treatments are compared, with average time duration of 17.42 seconds. In contrast, the seizure periods for chamomile and Ashwagandha are 21.68 s and 43.38 s, respectively. The combination yields the closest result of 17.42 s compared to Diazepam (14.1 s).

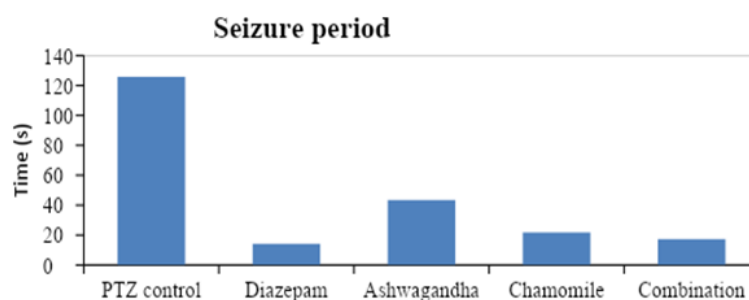


Fig 2: Seizure period of all treatments

Figure 2 is the graphical representation of the seizure period (the time between the first jerk and the end of the postictal depression, represents the point at which the animal becomes normal). When compared to other treatment groups and the PTZ control group, the combination has less time for a seizure period. But Diazepam, the reference control, showed better response. Regarding the seizure phase, the treatment groups experienced four to five jerks during the short clonic phase. Figure 3 shows the position of clonic seizure in mice where the forelimb and hind limb are contracted into one place. A Straub tail is observed, however

there were significantly more jerks in the PTZ control group. The animal collapses onto its side during a full tonic seizure (Figure 4), convulsing throughout its body. Only three of the animals in the combination group experienced a tonic seizure, and they recovered in less than ten seconds. However, it took the PTZ control group approximately fifteen seconds to recover from a tonic seizure. In the postictal depression period, which measures time taken for the animal to return to its normal state, but the combination group recovered in less than 8 seconds.



Fig 3: Position of Clonic seizure of mice

Figure 3 shows the position of mice in clonic seizures. During a clonic seizure, the tail bents, which is phrased as "Straub tail," and the limbs gets contracted to the center and pretend to be in a sitting position without moving, and some may have tremors during this position.



Fig 4: Posture of Tonic seizure of mice

Figure 4 shows the position of mice in a tonic seizure, where the animal collapses and falls on a side with full body contraction (lordosis spine). If the concentration of PTZ is high, the animal will have severe tonic seizures and will die in this period.

3.3. Effect of herbal compounds

Table 2 shows that both the herbal compounds Ashwagandha and Chamomile have effects against PTZ-induced epileptic seizures in swizz albino mice. So, comparing all treatments, Combination has better results with a seizure period of 17.42 s.

Table 2: Values of latency and seizure periods of individual groups.		
Group	Latency period (sec)	Seizure period (sec)
PTZ control	66±26.15	125.76±66.6
Diazepam	103.5±20.50	14.1±1.78
Ashwagandha	68.5±7.04	43.38±42.87
Chamomile	102.2±42.64	21.68±28.77
Combination	77.25±14.38	17.42±13.87

Table 2 illustrates the values of the latency period and seizure period of individual groups. A total of 5 animals were analyzed, and the mean and standard deviation of all 5 animals' latency and seizure periods were calculated and displayed.

3.4. Statistical analysis

Table 3 represents the P value and the significance at a 95% confidence interval for the latency period. All p-values exceed the 0.05 threshold, indicating no statistically significant differences between treatment pairs. Specifically, comparisons of PTZ with Diazepam, Ashwagandha, Chamomile, and the combination treatment all show non-significant p-values, as do comparisons of Diazepam with Ashwagandha, Chamomile, and the combination.

Table 3: P value and significance for the latency period		
Parameter	P value	Significance
PTZ vs Diazepam	0.6155	Ns
PTZ vs Ashwagandha	0.5558	Ns
PTZ vs Chamomile	0.1352	Ns
PTZ vs Combination	0.6515	Ns
Diazepam vs Ashwagandha	0.911	Ns
Diazepam vs Chamomile	0.1194	Ns
Diazepam vs Combination	0.8398	Ns
Ashwagandha vs Chamomile	0.0678	Ns
Ashwagandha vs Combination	0.909	Ns
Chamomile vs Combination	0.0742	Ns

Note: The number of “*” represents the level of significance. Ns – Not significant

Table 3 illustrates the P value and the significance between each group of the latency period. The latency period is between the administration of PTZ and the appearance of the first jerk. The significance was calculated at a 95% interval; no significance is shown in the table. The P value and significance at a 95% confidence range for the seizure period are shown in Table 4. Highly significant differences ($p \leq 0.01$) were observed between PTZ and Diazepam and the Combination treatment, indicating a substantial difference in their effects. Significant differences ($0.01 < p \leq 0.05$) were found between PTZ and Ashwagandha, PTZ and Chamomile, Ashwagandha and Diazepam, and the Combination and Diazepam, suggesting notable but less pronounced differences. PTZ and Chamomile, Ashwagandha and Diazepam, and the Combination and Diazepam suggest notable but less pronounced differences.

Table 4: P value and significance of seizure period		
Parameter	P value	Significance
PTZ vs Diazepam	0.0012	**
PTZ vs Ashwagandha	0.0485	*
PTZ vs Chamomile	0.0126	*
PTZ vs Combination	0.0042	**
Ashwagandha vs Diazepam	0.0335	*
Chamomile vs Diazepam	0.0862	Ns
Combination vs Diazepam	0.0125	*
Ashwagandha vs Chamomile	0.5823	Ns
Ashwagandha vs Combination	0.2449	Ns
Chamomile vs Combination	0.509	Ns

Note: The number of “*” represents the level of significance. Ns – Not significant

Table 4 illustrates the P value and the significance between each group in the seizure period. The seizure period is the time between the first jerk and the end of postictal depression (Animal returning to the normal stage). The significance was calculated at a 95% interval, and * $p < 0.05$ significance was observed in four parameters, and ** $p < 0.01$ significance was observed in two parameters.

4. DISCUSSION

One of the most prevalent and incapacitating neurological disorders is epilepsy; yet, its exact causes and, consequently,

reasons for treatment of most forms of epilepsy remain poorly understood.²⁷ Frequent, spontaneous seizures are referred to as “epilepsy.” Many factors contribute to epilepsy, and they are all indicative of underlying brain

dysfunction²⁸. Seizures can occur when there is a disturbance between excitation (E) and inhibition (I) in the brain. Many different aspects of brain function, including genes, intracellular signaling cascades, and extensive neural networks, can be altered to cause this E/I imbalance²⁹. The most well-known system in the CNS is the inhibitory GABA route, which can have abnormalities in function under various physiological circumstances. There are two kinds of GABA receptors. While GABA-B receptors are G protein-coupled potassium channels, GABA-A receptors are chloride ion channels³⁰. Commonly, there are some chemicals used to induce epilepsy, among which are Pentylenetetrazole (PTZ) and Pilocarpine. PTZ is a GABA-A receptor antagonist that reduces its activity noncompetitively. Pilocarpine causes damage to GABAergic neurons in the hippocampus by activating the cholinergic system in the brain³¹, which can cause seizures in animals^{32,33}. In this study, PTZ is used to induce seizures. The seizure stages, can be compared such as the latency period, clonic period, tonic period, and postictal depression. But for better understanding, the clonic period, tonic period, and postictal depression were combined as a seizure period. There are studies for the Anti-epileptic properties of *Cymbopogon citratus*³⁴, *Desmodium triflorum*²⁰, *Lophira alata*³⁵, *Mentha piperita*³⁶, *Melissa parviflora*³⁷, *Nepeta menthoides*³⁸, *Carissa edulis*³⁹, etc. There are also studies related to *Withania somnifera*⁴⁰ and *Matricaria recutita*⁴¹. In ²⁴, the concentration of PTZ used was 60 to 100 mg/kg b.wt, but here we used 120 mg/kg b.wt, which was comfortable in developing clonic-tonic seizure in the PTZ control group. The latency period encountered ⁸ for PTZ control is 84.13 s and 14.8 s ¹¹), where they used 80 mg/kg b.wt of PTZ, whereas, in our study, the latency period of PTZ control is 66 s in 120 mg/kg b.wt. However, the latency duration is relatively longer when chamomile is used. We observed that the seizure period was significantly shorter than all the treatments in our study, where they used *Desmodium Triflorum* for evaluation. However, the latency period ²⁰ is

consistent with our findings in PTZ control and treatments. A longer tonic and less latency period, using only 35 mg/kg b.wt of PTZ was reported⁴². diazepam, which was used as the reference control, only one or two jerks were observed, and in only one animal, we encountered full tonic seizures, almost every study that used Diazepam as reference control^{43,45} showed full protection against PTZ convulsions^{20,45}. Phenytoin is also used as reference control^{37,40}. Further evaluation shall be made by increasing the dose of both herbs in higher species.

5. CONCLUSION

In conclusion, the study aimed to evaluate the anti-epileptic properties of *Withania somnifera* and *Matricaria recutita* extracts on PTZ-induced seizures in Swizz albino mice. The results indicated promising anti-epileptic activity of the herbal extracts, with chamomile showing a longer latency period and the combination group exhibiting favorable results during the seizure period. Further work will be done by increasing the dose of both herbs.

6. AUTHORS CONTRIBUTION STATEMENT

Dr. P.S. Venkatesan, the Principal investigator, conceptualized and designed the study and monitored Co-investigators' activities. He verified the data and finalized the article. M.Eswarya, the Co-investigator, dosed the animals and recorded the clinical signs and the data. She compiled the data and was also involved in article preparation. M.Madhavaselvi supervised the animal house activities, participated in data recording, article writing, and reference collection, and contributed to the project's financial support.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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